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Heparin – An old drug with multiple potential targets in Covid-19 therapy

To the Editor,

A prominent clinical feature of severe Covid-19 infection is respiratory failure associated with pulmonary coagulopathy. Recent reports published in the *Journal of Thrombosis and Haemostasis* show that treatment with low molecular weight heparin (LMWH) decreases mortality in critically ill patients with sepsis-induced hypercoagulation, and thus argue for prophylactic administration of the anticoagulant.[1-4] In addition, the authors point to non-anticoagulant activities of heparin, in particular anti-inflammatory effects with potential to prevent deterioration of the disease. We would like to use this opportunity to clarify the biochemical background of the diverse activities of heparin, and, further, how this information may be exploited to generate more efficient treatment of the viral infection. Mechanisms to consider relate to the functional roles of proteins interacting with heparan sulfate (HS), a polysaccharide closely related to heparin.

The highly sulfated glycosaminoglycan, heparin, has been used as an antithrombotic since the 1930s, and remains one of the most widely prescribed drugs today. The polysaccharide, a product of mast cells, is isolated from animal tissues (pig intestinal mucosa, bovine lung), and owes its anticoagulant activity to a specific pentasaccharide sequence (Figure 1A) that binds and activates antithrombin (AT), an inhibitor of proteases involved in blood coagulation. Only a fraction of the polysaccharide chains in heparin preparations contain the AT-binding pentasaccharide structure and hence bind AT with high affinity (HA-heparin) and show significant anticoagulant activity.[5] The low-affinity chains (LA-heparin) have low anticoagulant activity but otherwise polyanionic properties similar to those of HA-heparin. The partially depolymerized LMWH preferentially used in the clinic contains about 30% HA chains.

Contrary to heparin, HS is expressed by virtually all mammalian (and many nonmammalian) cells, in particular as cell-surface proteoglycans with HS chains exposed to the extracellular milieu. Heparin and HS contain the same saccharide building blocks. However, although heparin is largely composed of extended, heavily sulfated saccharide sequences, HS displays distinct domains, the sulfation patterns of which are regulated in a cell-autonomous fashion (Figure 1A,B). The negatively charged domains serve to bind a multitude of proteins, including growth factors and their receptors, cytokines, selectins, extracellular-matrix molecules, certain viral coat proteins, and others. Some proteins bind selectively to cognate HS sequences, whereas others show less specificity.[6] Virtually all HS-binding proteins also interact with heparin, hence the term "heparin-binding proteins" (Figure 1C).

Following administration to patients, heparin will not only activate AT, but may also affect the functional state of a variety of other proteins. By competing with cell-surface HS for protein binding, heparin will displace proteins from their HS-mediated anchoring and thus disrupt associated function. Effects of potential value in Covid-19 treatment include prevention of viral adhesion but also anti-inflammatory activity based on inhibition of neutrophil chemotaxis and leukocyte migration. The recurrent involvement of proteins bound to cell-surface HS is striking. Binding of a viral protein to cell-surface HS is often the first step in a cascade of interactions that is required for viral entry and the initiation of infection. Indeed, recent findings of heparin interacting with the receptor binding domain of the SARS-CoV-2 Spike S1 protein suggest the potential to

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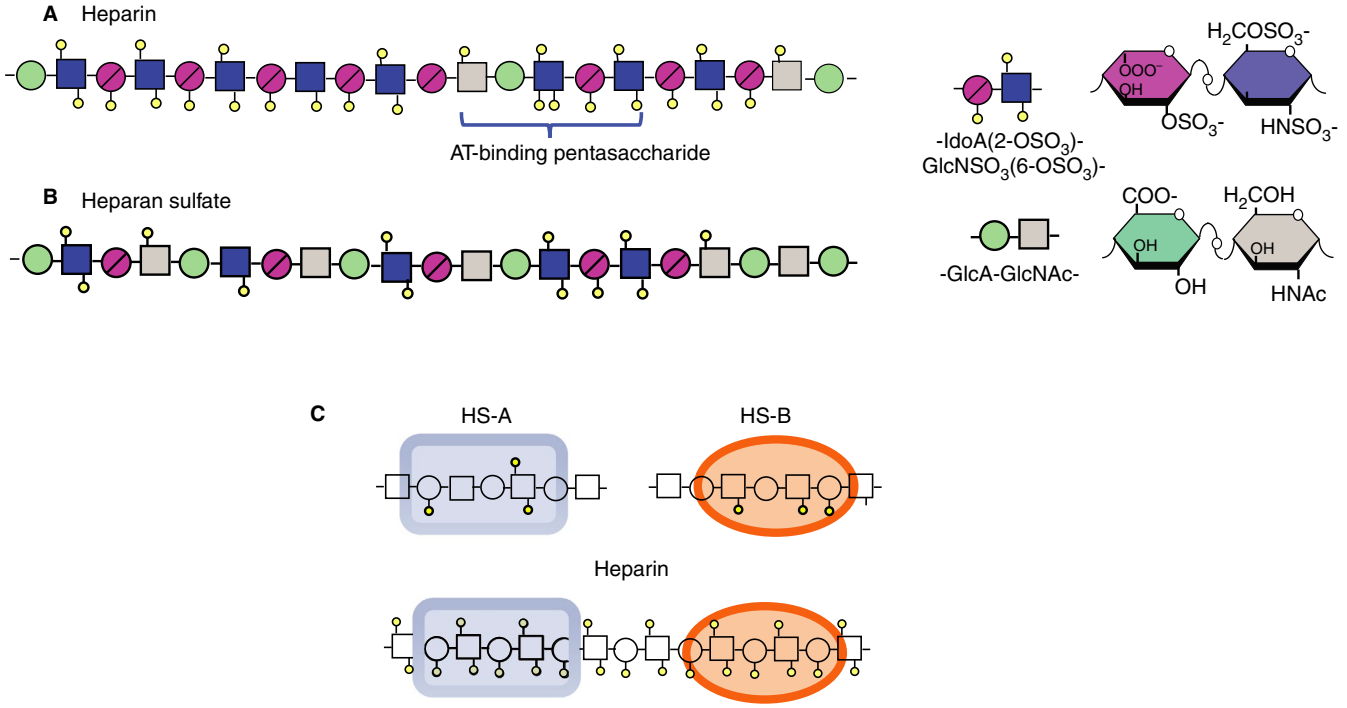

FIGURE 1 The structural/functional relationship between heparin and heparan sulfate provides the following. (A) Conjectured heparin chain composed of alternating units of hexuronic acid (D-glucuronic acid, GlcA, or L-iduronic acid, IdoA) and D-glucosamine (GlcN); sulfate groups are indicated by yellow circles. The AT-binding pentasaccharide sequence carries a unique sulfate group at carbon 3 of the internal GlcN residue; this 3-O-sulfate group is present in HA- but not in LA-heparin.[5] (B) HS structure, composed of the same building blocks as heparin, but with more sparse distribution of IdoA and sulfate residues. These components are typically accumulated in more densely sulfated domains that vary in structure depending on cellular origin. The sulfated domains provide sites for interactions of varying specificity with a multitude of proteins.[6] (C) Schematic illustration of two proteins with distinct requirements for binding structures on HS chains, yet both interacting with heparin. Rather than nonspecific, such interaction may depend on "hidden specificity," the selective HS epitopes being expressed also in the heavily sulfated heparin chain, albeit masked by redundant sulfate groups

prevent viral adhesion.[7] Interactions of selectins and cytokines with HS expressed on endothelial cells control the recruitment of immune cells during inflammation.[8] Notably, in a retrospective clinical study, LMWH treatment of COVID-19 patients was found to significantly lower plasma levels of interleukin-6, a key player in the "cytokine storm" associated with severe outcome of the disease.[9] Potentially beneficial effects of heparin on initiation and progression of Covid-19 infection would thus be due to displacement of key protein protagonists from their HS scaffolds.

These considerations argue for increased attention to the non-anticoagulant properties of heparin in Covid-19 treatment, particularly because the inflammatory phase is likely to precede the development of pulmonary microthrombi and impaired pulmonary gas exchange. However, due to the risk of bleeding complications the anticoagulant activity sets a limit to dosage of LMWH. In theory, this problem could be overcome by increasing the proportion of LA components, that are likely to harbor the protein-binding properties required. Unfortunately, pure LA-heparin is not available as a distinct drug. Instead, there are several reports of procedures to selectively eliminate the anticoagulant activity from heparin, without affecting the overall polyanionic properties.[10] We suggest that such products, that can be applied at higher dose, should be considered as a complement to conventional LMWH.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

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Clinical differentiation of anticoagulant and non-anticoagulant properties of heparin

I thank Lindahl and Li for their thoughtful comments on the non-anticoagulant properties of heparin.[1] Heightened awareness of hypercoagulability has made heparin part and parcel of the COVID-19 management algorithms. In addition, reports of prophylactic anticoagulation failure have triggered several trials in which escalated doses of heparin are compared with standard doses with the aim of preventing thrombotic complications. At this juncture, we do need to consider where do the non-anticoagulant properties of heparin fit in the COVID-19 clinical context?

As the letter suggests and based on several experimental studies, heparin has several non-anticoagulant properties that are likely to benefit symptomatic COVID-19 patients. These include anti-inflammatory, anticomplement, antiplatelet, and endothelial quiescence in addition to even being antiviral.[2,3] But, we do have to bear in mind that many of these data are from research laboratories and have not found their way into mainstream clinical use. This may be due to a lack of awareness of these various "outside the box" properties of heparin. Also, these functions are always considered as ancillary to the anticoagulant role rather than considered on their own accord. In other words, the non-anticoagulant properties are only considered in patients who are anticoagulated for proven thrombosis or having high risk for thrombosis. In addition, concerns of bleeding dissuade heparin use in patients with an increased risk of bleeding—for example, critically ill septic patients—which are exactly the patients for whom the non-anticoagulant properties may have an important role. Heparins without anticoagulant function (heparin analogues) may be the answer here[3] but once again we need proof of efficacy in clinical studies. Until then, we have to continue using conventional heparin, while trying to identify ways to demonstrate the non-anticoagulant effects of heparin clinically.

One of the well-known complications of COVID-19 is acute lung injury, caused by the virus itself and virus-induced inflammation. Activated leukocytes in the setting of inflammation degrade glycocalyx, the physiological endothelial protective barrier.[4] This allows transmigration of the cells relevant to the inflammatory process causing vascular leakage, the severity of which can correlate with the degree of hypoxemia.[4] In experimental animals with sepsis, both unfractionated and low molecular weight heparins abrogated this complication.[5] The clinically relevant question here is when should we intervene with heparin because inflammation in the early stages is clearly a host defence mechanism, but, if continued unabated, could lead to irreversible damage. The early, physiological response is clearly part of host defence and unlikely to be harmful but the latter stages, during which the markedly increased vascular leakage has stopped adequate gas exchange, would be pathological. Hence, considering heparin administration at early signs of hypoxia may be the most appropriate time for exploiting its anti-inflammatory, protective function. It would of course be ideal to have biomarkers which can "detect" the increased vascular leakage to aid appropriate timing of heparin commencement.

It's not just the wrong timing behind the failure of heparin trials for acute respiratory distress syndrome (ARDS) effectiveness,[6] but

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